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Site fertility and soil water-table level affect fungal biomass production and community composition in boreal peatland forests

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Originality-Significance Statement

We will confirm that all the reported work in the manuscript is original and has not been published elsewhere. Neither is it being considered for publication elsewhere. All authors have seen the final version of the manuscript and accepted it to be submitted for publication in Environmental Microbiology.

This study increases the fundamental knowledge on fungal biomass production potential and fungal community composition of both symbiotic ectomycorrhizae and saprotrophs in boreal peatland forests with varying fertility from nutrient-rich to nutrient-poor types. The obtained results give indications that specific ectomycorrhizal fungi would be key factors affecting the fungal biomass production and persistence which is affected by both site fertility and water-table level of the studied peatland forest sites. The manuscript is rather long, but we feel that it is justified because the backbone is built from the large and diverse data set. We did not want to discard any data or results, since we feel that they complement each other and together make more consistent story. We would place the work within the top 10% of current research in environmental microbiology.

On behalf of all authors,

Dr. Krista Peltoniemi (Corresponding author)

Summary

A substantial amount of below-ground carbon (C) is suggested to be associated with fungi, which may significantly affect the soil C balance in forested ecosystems. Ergosterol from in-growth mesh bags and litterbags was used to estimate fungal biomass production and community composition in drained peatland forests with differing fertility. Extramatrical mycelia (EMM) biomass production was generally higher in the nutrient-poor site, increased with deeper water table level and decreased along the length of the recovery time. EMM biomass production was of the same magnitude as in mineral-soil forests. Saprotrophic fungal biomass production was higher in the nutrient-rich site. Both ectomycorrhizal (ECM) and saprotrophic fungal community composition changed according to site fertility and water table level. ECM fungal community composition with different exploration types may explain the differences in fungal biomass production between peatland forests. Melanin-rich *Hyaloscypha* may indicate decreased turnover of biomass in nutrient-rich young peatland forest. Genera *Lactarius* and *Laccaria* may be important in nutrient rich and *Piloderma* in the nutrient-poor conditions, respectively. Furthermore, *Paxillus involutus* and *Cortinarius* sp. may be important generalists in all sites and responsible for EMM biomass production during the first summer months. Saprotrophs showed a functionally more diverse fungal community in the nutrient-rich site.

Introduction

Peatlands are one of the major reserves of soil carbon (C) globally, with total peat C stocks in northern peatlands alone estimated to range from 545 Gt to 1,055 Gt (Nichols and Peteet, 2019). Widespread drying of peatlands has been observed as a response to climate change and human activities (Swindles *et al*., 2019), which will shape the peatland C sink (Gallego-Sala *et al*., 2018). Drying, both climate-driven (Berg *et al*., 2009) and anthropogenic (Laiho *et al*., 2003), lead to an increased area of peatland forests. In drained peatland forests especially, water-table level is relatively low and leaves a larger proportion of the peat deposit oxic. This increases microbial decomposition and respiration, with the end product, carbon dioxide (CO_2), being released into the atmosphere and the accumulated soil C reservoir of the peatland decreasing in many, but not all cases (Ojanen *et al.*, 2013; Minkkinen *et al.*, 2018). The soil C stock generally decreases in nutrient-rich but not nutrient-poor peatland forests, even though litter input to the soil may be higher from faster-growing and thus generally larger tree stands in the rich sites (Ojanen *et al*., 2013). The constraints for this difference are not well known (e.g., Linkosalmi *et al.*, 2015), though further research may explain the resilience of the soil C stocks in these areas.

Fungi are generally key organisms in regulating forest soil C dynamics, and a major part of soil C in boreal mineral-soil forests is derived from roots and root-associated fungi (Clemmensen *et al.*, 2013). While saprotrophic fungi take up nutrients in the decomposition process, root-associated mycorrhizal fungi recycle them back into their host plants. In exchange, trees serve to their symbiotic fungal partner fresh C. In general, plants are estimated to allocate 10–20% of net photosynthate to mycorrhizal fungi, although the range can be from 5 to 85% depending on the system (reviewed by Allen, 1991). Thus far, there is little information available on fungal communities and processes in peatland forests, despite their importance as soil C hotspots.

To be able to estimate the role of fungi in soil C sequestration in peatland forests, we must know the potential of fungi to produce biomass in these habitats, and what are key environmental factors

affecting the production. The soils in peatland forests differ fundamentally from those of forests on mineral soils. Peat soil is an organic soil consisting of various plant materials in varying degrees of decomposition, depending on the recent and past plant communities and the moisture regime, which is an integral regulator of both the plant community and rate of decomposition (e.g. Belyea 1996, Bragazza 2006; Hájek *et al.*, 2013). All peat soils contain a high amount of nitrogen, varying between 0.5 and 4% of dry mass (Laine et al. 2004), compared to mineral soils, but little phosphorus and potassium (e.g., Westman and Laiho 2003). This may affect the balance between saprotrophic and mycorrhizal fungi (e.g., Lindahl *et al*., 2007), but there is currently no information available for peatland forests. Most of the studies estimating extramatrical mycelia (EMM) biomass produced by ectomycorrhizal (ECM) fungi have been conducted in mineral-soil forests. For example, Ekblad *et al.* (2016) showed that nitrogen (N) fertilization reduced the standing EMM biomass. In boreal peatland forests, increased EMM biomass was observed in response to both P and K deficiency (Potila *et al.*, 2009) and due to fertilization with a mixture of apatite and iron phosphate (Nieminen *et al.*, 2011) in which P is not easily available but in a slow-releasing form. Thus, we expect that site fertility of drained peatland forests would be one of the major factors impacting fungal biomass production.

Different fungal species, however, may have different capacity to produce biomass. Therefore, along with the biomass, it is important to study the fungal community composition. In boreal mineral-soil forests, soil fungal community composition has been found to be significantly related to site fertility (Toljander *et al.*, 2006; Sterkenburg *et al.*, 2015). Also, in boreal peatlands site fertility has been shown to be one of the key determinants of the soil fungal community (Jaatinen *et al*., 2007; Peltoniemi *et al*., 2009). Fungal specific phospholipid fatty acids (PLFAs) in peat decreased with the fertility in pristine sites, but after drainage differences between sites were not so evident anymore (Jaatinen *et al*., 2007), whereas soil fungal community composition became more similar (Peltoniemi *et al.*, 2009).

Analyses conducted directly from soil, however, cannot separate the biomass or communities between mycorrhizal and saprotrophic fungi. Therefore, we used two separate methods to determine fungal biomass production and community composition for these functionally different groups:

"Traditional" sand-filled in-growth mesh bags for EMM, and litter-filled bags for saprotrophic fungi. The in-growth mesh bag method has been designed for forest soils to estimate the biomass of EMM from ECM fungi (Wallander *et al.,* 2001). EMM estimations are most often based on fungal specific membrane lipid ergosterol, which is extracted from fungal hyphae grown into mesh bags (Wallander *et al*., 2013). Ergosterol content is suggested to correlate with the amount of metabolically active fungal biomass (Nylund and Wallander, 1992) and is used as an estimate for total fungal biomass. The ratio of chitin to ergosterol has been used as an estimate for the living fraction of fungal biomass (Ekblad *et al*., 1998). In this study ergosterol was used with chitin to determine the living fungal fraction left in the sandbags after 12 months.

Fungal biomass production and degradation in soil is a dynamic process. Therefore, it is difficult to estimate the absolute turnover of fungal biomass. The mesh-bag method has of course limitations and deficiencies as every method. It has been shown that fungal communities in the bags may change and shifts in mycelial exploration types may occur depending on the stand age or if bag incubation time exceeds 75 days (Hagenbo *et al*., 2018). The mesh bag method, however, enables relative comparisons of biomasses and estimations of EMM production over time (Hagenbo *et al*., 2017). Here, we compare fungal biomass production and community composition in boreal drained peatland forests and reference mineral-soil forests differing in site fertility (nutrient-rich versus nutrient-poor). For estimations of biomass production of ECM fungi, we used ergosterol extracted from fungal mycelia obtained from sand-filled mesh bags recovered at different time points; after 2, 5 and 12 months. We determined biomass production of saprotrophic fungi from ergosterol extracted from litterbags containing two different plant materials, corn and wheat, that were placed in trenched locations to exclude non-saprotrophic fungi, and non-trenched locations for comparison. We determined fungal community composition from extracted DNA obtained from hyphae grown inside the sand- and litterbags. We assessed the following hypotheses: 1) nutrient-rich peatland forests have lower EMM biomass production compared to nutrient poor sites, 2) ECM fungal community composition changes along with site fertility, 3) recovery time of the sandbags has impact on both EMM biomass production

and ECM fungal community composition results, and 4) saprotrophic fungal biomass production and community composition differs between nutrient-rich and poor sites.

Results

Fungal biomass production and the amount of living fungal biomass

The overall average EMM biomass production in sandbags for peatland forests was 34.1 (SE± 3.7) kg ha^{-1} $month^{-1}$, and for mineral-soil forests 32.7 (± 4.8) kg ha^{-1} $month^{-1}$ (Fig. 1a); there was thus no overall difference between soil types. The production in peatlands depended on site type, average WT during the recovery time, length of the recovery time, and soil N:P ratio, which altogether explained 41.6% of the total variation (Table 2). Production was higher in the nutrient-poor sites, increased with deeper WT, decreased with a higher N:P ratio, and decreased with the length of the recovery time in a somewhat non-linear manner (Table 2). Recovery time alone explained 22.9% of the variation, while site type and WT together explained 30.4%; alone these two variables were not significant. Burying depth alone explained only 1.3%, and N:P ratio effect alone was only barely significant. The impact of N:P ratio was caused by changes in P concentration, with which EMM had a positive relation almost as strong as the negative one with N:P, while switching to N concentration in the model yielded a non-significant parameter. The amount of living fungal biomass in the sandbags after 12 months was biggest in Rich1 site and differed significantly from that of Rich2 and Poor2 sites (Supporting Information Fig. S1). Site-specific glucosamine and ergosterol amounts and glucosamine:ergosterol-ratios can be found in Supporting Information Table S2.

The average fungal biomass production in the litterbags was 11.8 (SE±11.5) mg g^{-1} litter dry mass $month^{-1}$ (Fig. 1b). The production varied by litter type, which alone explained 26% of the variation, while depth explained 21% and site 10%. Production was higher in the nutrient-rich site, upper soil layer, and corn litter (Fig. 1b). Trenching was not significant as a main effect. However, when

interactive effects were evaluated, trenching was found to have a significant interaction with site, indicating that trenching effects differed between sites. A closer look revealed that in the deeper layer of the nutrient-poor site trenching reduced fungal biomass production in both litter types, while in the nutrient-rich site, trenching had no impact in the deeper layer (Fig. 1b). Variation in the litterbag data was best explained by a model combining site x trenching interaction, litter type, burying depth of the bags, which altogether explained 65% of the variation (Table 3).

General information of the obtained fungal sequences

Altogether, 12 371 497 raw reads from 108 sandbag sample libraries and 6 289 946 from 48 litterbag sample libraries were obtained. Average read length was 326 bp. A total of 5 306 393 reads from the sandbag libraries and 1 927 262 from the litterbag libraries were retained after screening. Library sizes in sandbags varied from 4 586 to 103 258 reads with an average of 49 133 (± 20 157). Library sizes in litterbags varied from 8627 to 75 993 reads with an average of 40 151 (± 14 118). Sequence data was classified into 2 938 and 940 different fungal OTUs from sand- and litterbags, respectively. Combined OTU data with unique fungal representatives contained 1 142 and 436 OTUs from sand- and litterbags, respectively.

Of the sandbag derived fungal OTUs, 51% represented Ascomycota, 40% Basidiomycota, 7% seven other phyla (Chytridiomycota, Entomophthoromycota, Glomeromycota, Mortierellomycota, Mucoromycota, Olpidiomycota, Rozellomycota) and 2% remained unidentified. Of the litterbag derived fungal OTUs, 57% represented Ascomycota, 33% Basidiomycota, 8% five other phyla (Chytridiomycota, Mortierellomycota, Mucoromycota, Olpidiomycota, Rozellomycota) and 2% remained unidentified.

Fungal community composition of the sandbags

Fungal community composition in the sandbags differed clearly between sites and formed a clear gradient from the Rich1 peatland forest to PoorM mineral-soil forest (Fig. 2). The significant environmental variables, soil pH, C N, P and Fe concentrations, C/N-ratio and WT, indicated that both nutrient and moisture regimes had an impact on the variation in fungal community composition in peat. Overall, site had the strongest effect on fungal community composition of the sandbags, explaining 16 % of the variation (Supporting Information Table S3). Recovery time of the bags and sampling depth both explained 3%. Interactive variables site x recovery time and site x depth explained 10 % and 5 % of the variation, respectively, indicating that the time and depth effects were partly site dependent.

Fungal richness (assessed by OTU numbers) was higher in Rich2 and RichM compared to the Poor1 and PoorM sites, 15-30 cm depth compared to 0-15 cm depth, and after 2 months compared to 5 and 12 months (Supplementary figure S2). There were 20, 16 and 17% shared fungal representatives (total of 1142 OTUs) between sites after 2, 5 and 12 months (Supporting Information Fig. S3). Of the shared fungal representatives, 49, 55 and 57% after 2, 5 and 12 months, respectively, belonged to the dominant fungal genera. The proportion of unique representatives specific for each of the six sites varied from 1 to 7 %. Generally, there were more unique fungal representatives in the lower 15-30 cm soil horizon. Fungal representatives from sandbags were grouped into 94 different functional guilds. ECM fungi were the most abundant group in peatland forests, with their proportion ranging from 56 to 84% depending on site and recovery time (Supporting Information Fig. S4). In mineral-soil forests, the proportion of ECM fungi was higher in RichM (56 – 67%) compared to PoorM (27 – 45%)

Dominant fungal representatives (OTUs) grouped into 37 different taxa including common ECM fungal genera, e.g., *Tylospora, Paxillus, Suillus, Russula, Tomentella, Tomentellopsis, Laccaria, Lactarius* and *Piloderma* (Fig. 3). Differential abundance analyses showed that there were various ECM fungal representatives that had significant changes in their OTU abundances between compared sites (Supporting Information Table S4). For example, sequence reads affiliated to *Hyaloscypha bicolor*, *Laccaria* sp., *Lactarius* sp., *Lactarius rufus* and *Russula nitida* were more common in the Rich1 compared to the other peatland sites. Sequence reads affiliated to *Cortinarius lux-nymphae*,

Amanita fulva and *Tomentella stuposa* were more common in the Rich1 site compared to the two Poor peatland sites. In contrast, sequence reads affiliated to *Suillus variegatus*, *Tylospora* sp. and *Piloderma sphaerosporum* were more common in both Poor sites compared to the Rich peatland sites. In addition, some ECM fungal representatives were common in some but not all recovery times of the bags. For example, sequence reads affiliated to *M. bicolor* were more common in the bags after 2 and 12 months than after 5 months; by 21 and 15 times more, respectively. Representative reads affiliated to *Tomentellopsis* sp. were more common after 5 months than after 2 or 12 months; by 5 and 4 times more, respectively. Representatives of *Cortinarius lux-nymhae* and *Tylospora* sp. were more common after 12 months than after 2 months, and those of *Cenococcum geophilum* were more common after 2 and 5 months compared to bags after 12 months; by 2 and 4 times more, respectively.

Considering the individual soil characteristics, pH and C/N-ratio correlated most strongly with the abundance of specific sequence reads. For instance, the abundance of sequence reads affiliating to species *Tomentellopsis submollis, Suillus bovinus,* and *L. rufus* increased with increasing pH, while the abundance of reads affiliating to *Suillus variegatus*, three *Russula* species, *Paxillus involutus*, *Piloderma sphaerosporum* and *Tylospora* decreased with increasing pH (Supporting Information Table S6). The abundance of sequence reads affiliating to, e.g., *Piloderma sphaerosporum, Russula paludosa* and *Suillus variegatus* increased with increasing C/N-ratio, while the abundance of reads to *Russula nitida, Paxillus involutus, Atheliaceae, Lactarius necator, L. rufus* and *Piloderma* sp. decreased with increasing C/N-ratio.

Fungal community composition of the litterbags

Fungal community composition in litterbags clearly differed between the Rich1 and Poor1 sites (Fig. 4). Site explained 7 % of the variation in the fungal composition (Supporting Information Table S3). Sampling depth, litter type and trenching treatment explained 8, 7 and 4 % of the variation in the fungal community composition in the litterbags, respectively. There were several interactive effects of site,

depth, litter type and/or treatment, but they all explained only a minor part (1– 4 %) of the variation, and almost half (48 %) of the variation remained unexplained.

Fungal richness (assessed by OTU numbers) did not differ between sites, depths or litter types, but it was higher in control compared to trenching (Supplementary figure S2). About 80% of the fungal OTUs (total of 436 OTUs) were shared across sites, depths, litter types and trenching treatment (Supporting Information Fig. S5). Site specific unique fungal OTUs affiliated to 46 and 24 different fungal genera and grouped into 21 and 12 functional fungal guilds in the Rich1 and Poor1 sites, respectively. In addition, corn litter compared to wheat, the lower 15-30 cm sampling layer compared to the 0-15 cm layer, and trenching compared to control, had lower amounts of specific functional fungal guilds (data not shown). The litterbag OTUs were grouped into 64 different functional guilds. About half of the reads belonged to undefined saprotrophs and over 20 % remained unknown for their ecological function (Supporting Information Fig. S6).

The dominant fungal representatives in the litterbags affiliated to 26 different taxa including, e.g., genera *Apiotrichum, Ascococryne, Botrytis, Gymnopilus, Hypholoma, Hypochnicium, Hyaloscypha, Mortierella, Mucor* and *Sugiyamalla* (Fig. 5). Almost half of the dominant taxa, 46 %, affiliated to saprotrophic fungal guilds, 27 % affiliated to guilds having mixed roles or combination to act as pathogens, saprotrophs or symbiotrophs, 23 % were not identified to any known functional guilds and only 4 % were pure pathogens. Trenching decreased the proportion of ECM fungi by 40 % after even though in general the proportion was very low (data not shown). Differential abundance analysis showed that there were various fungal representatives that had significantly different abundance either between sites, sampling depths, litter types or treatments (Supporting Information Table S6). For example, there were 40 and 46 fungal indicators to Rich1 and Poor1, respectively. OTUs affiliating to *Trichoderma fertile*, *Pseudeurotium bakeri*, *Ascobolus* sp., *Hypochnicium albostramineum* and *Gyoerffyella* sp. were more common in the Rich site, whereas those affiliating to taxa Pyronemataceae and *Gymnopilus decipiens* were more common in the Poor site. *Ascobolus* sp. were more common in the 15-30 cm layer, in wheat litter and in control plot without trenching. Pyronemataceae was more

common in the 0-15 cm sampling layer, in corn litter and after trenching. *Pseudeurotium bakeri* was more common in the 15-30 cm sampling layer and in corn litter.

We detected several strong and moderate positive and negative correlations between the abundance of 22 dominant fungal representatives and soil pH, nutrients, C/N-ratio and fungal biomass in the litterbags (Supporting Information Table S7). For example, *Apiotrichum xylopini* and *Hypochnicium albostramineum* showed positive correlation with soil pH, total N and C content, and iron (Fe) concentration and negative correlation with C/N-ratio. In contrast, *Hyaloscypha variabilis, Sugiyamaella paludigena, Sordaria fimicola, Mucor silvaticus* and *Trichoderma viride* had negative correlations with pH and N content, and positive correlation with C/N-ratio. Fungal representatives affiliating to *Hyaloscypha variabilis* and *Sugiyamaella paludigena* also had negative correlations with phosphorus, potassium, copper, boron, manganese, calcium, magnesium and zinc concentrations.

Discussion

EMM biomass production differed with site fertility

This was the first study to our knowledge to estimate fungal biomass production and community composition in a range of peatland forest sites and compare them with mineral-soil forests. Our results indicate firstly that EMM production is an equally important component in ecosystem function in peatland forests as in forests on mineral soils. They also indicate that the in-growth method works equally well in forests on peat and mineral soils, even though we cannot fully reject the possibility that mineral matter in the bags attracts EMM growth dissimilarly to the ambient organic soil in peatlands. Overall, the results were in range with earlier studies when considering the length of the recovery time of the bags (Potila *et al*., 2009; Yiyang *et al.* unpublished data). Our results suggest that the EMM production and community composition of ECM fungi in peatland forests are quite strongly determined

by the site nutrient regime, which can be described by floristically defined site type, and the depth of the soil water-table level (WT).

The overall effect of site type was higher EMM production in the nutrient-poor sites, supporting our hypothesis 1. This indicates that it is beneficial for ECM plants to invest more into supporting EMM production to harvest nutrients in low-nutrient sites, which appears logical and has been shown before for forests on mineral soils (Nilsson *et al.*, 2005). It is somewhat more intriguing that production further increased with decreasing N:P ratio or increasing P concentration in the soil. Fertilization studies (e.g. Paavilainen, 1980; Pietiläinen *et al.*, 2005) indicate that tree growth in peatland forests may be limited by N in sites similar to our Poor sites, but rather by P and/or K in Rich sites. Thus, it would seem more logical if EMM production increased with increasing N:P ratio, indicating investment in EMM to harvest P. In earlier studies higher EMM production at high N and low P and K conditions have been detected in both mineral-soil (Wallander and Nylund, 1992; Ekblad *et al*., 1995) and peatland forests (Potila *et al.*, 2009). Thus, we have no explanation currently for the opposite pattern, which seems to indicate that trees can "afford" investing into EMM when more P is available relative to N. However, fungal community composition may offer at least two partial explanations; ergosterol content is known to vary between fungal species (Baldrian *et al.*, 2013) and fast-growing species may comprise a major part of the biomass in mesh bags (Wallander, 2006).

Since the nutrient and WT regimes varied independently, the site type impact is not visible when looking at the production values between the individual sites. Clearly, production was strongly modified by the depth of the WT in the soil. This makes sense, since lower WT generally means a thicker soil layer under oxic conditions, as opposed to the anoxic conditions prevailing below the WT. Accordingly, the highest production values were determined from the Rich1 site where WT was deepest. WT is in general a strong constraint for ecosystem structure and function in peatlands (e.g. Mäkiranta *et al.*, 2018), and thus always needs to be considered to facilitate ecologically meaningful interpretation of any results for peat soils.

Supporting hypothesis 3, EMM biomass production seemed to follow the recovery time of the bags, i.e., its rate decreased over time. The results likely refer to a natural seasonal turnover process as has been also reported by Wallander *at al.* (2001) where the fungal biomass colonization peaks in the growing season and ceases in the winter period and is thus not recognized as production in longer recovery time. The higher amount of living fungal biomass after 1 year in sites Rich1 and Poor1 as compared to the other two peatland sites needs further investigation. However, compared to old tree stock in Rich2 and Poor2 sites, tree stocks in Rich1 and Poor2 are still young and growing well. In mineral forest soils, well-growing young stands are shown to have more actively growing EMM fungal communities (Kalliokoski *et al*., 2010). Thus, it seems that the ectomycorrhizal community produces biomass rapidly during the first summer months, and these two sites, especially the N-rich, can retain much of the fungal cells as living after the winter period. This may indicate differences in turnover times of EMM in sites of varying fertility, or alternatively due to the presence of a resilient fungal community in nutrient-rich sites.

Sampling depth alone did not have an impact on EMM production. Even the community was not very different, as discussed below. Also, in our previous study on a WT gradient, fungal biomass, estimated based on PLFAs and assumed to be mostly ECM, was relatively high in the deeper layers of the driest location (Jaatinen *et al*., 2008). This would indicate that ECM fungi can grow equally well in the deeper layer as in the surface layer in organic soils, and the closer proximity to the WT was not impacting them negatively.

Fungal richness and unique fungal representatives in sandbags

In contrast to EMM biomass production, fungal richness in sandbags was not higher in nutrient-poor sites. About half of the common fungal genera in all sites belonged to the dominant ones, and many of the ECM fungal species common in nutrient-poor sites were belonging to long exploration type fungus, implying a potential to produce greater amount of EMM biomass (Agerer, 2001). Thus, it seems that

the specific fungal signature of each site was contributed to by only a few fungi unique to each specific site. ECM fungi harvesting sandbags seemed to work equally well in the peatland as in the mineral soil forests, since the proportions of ECM fungi were similar or even higher in the peatland sites. Also, most of all dominant genera affiliated to ECM fungi.

Both fungal richness and the number of unique fungal OTUs were higher in the lower soil horizon. The result is consistent with the findings of Lindahl *et al.* (2007) from mineral-soil forest that mycorrhizal fungi dominated in the underlying, more decomposed soil. Like biomass, fungal OTU numbers indicate higher richness after 2 months, further suggesting that the more diverse fungal community is able to produce a large amount of fungal biomass rapidly in the very beginning of the growing season.

Site determines ECM fungal community composition

Supporting hypothesis 2, site type was a strong predictor of the fungal community composition in sandbags. The composition nicely followed a fertility gradient from the most nutrient-rich peatland forest to the nutrient-poor mineral-soil forest. Fungal communities in boreal forests on mineral soils are also known to follow the fertility gradient (Toljander *et al*., 2006; Sterkenburg *et al*., 2015), and our results indicate that peatland forests follow the same pattern surprisingly well. Sampling depth and recovery time explained much less of the fungal community composition, yet still supported hypothesis 3.

The results suggest fungal indicator taxa that are typical of a specific peatland forest type. Many of the indicative taxa in nutrient-rich sites were short or medium exploration type, such as *Laccaria* which was clearly more common in the Rich1 site compared to other sites. Genus *Laccaria* is a known r strategist which is capable of rapid mycelial growth, has low carbohydrate demand and is highly competitive (Dighton and Mason, 1985), and found abundantly from fertilized *Sphagnum*-peat growing media used in forest nurseries (Flykt *et al*., 2008). It appears that species of *Laccaria* would benefit

from nutrient-rich conditions from the very beginning of the growing period through the winter. On the other hand, the genus *Lactarius* was also significantly more abundant and common especially in deeper layers in Rich1.

Interestingly, fungal representatives affiliating to *Hyaloscypha bicolor* were also much more common in the Rich1 site compared to other sites and appeared more common in the beginning of the growing period (after 2 months) and then again after the winter (after 12 months). *H. bicolor* is known to form short exploration type mycelia and associate with both ericoid and ectomycorrhizal hosts (Grelet *et al.*, 2016). Species of *Hyaloscypha* (former *Meliniomyces*) have high melanin content in their cell walls, and melanized fungal necromass has been reported to decompose slowly (Fernandez *et al.*, 2013; Fernandez and Koide, 2014) and contribute significantly to C accumulation in soil organic matter (Clemmensen *et al*., 2015). However, the effect seems to be ECM species-specific as in our study the proportion of another melanized ECM fungus, *Cenococcum geophilum*, decreased with recovery time. Later, Fernandez and Kennedy (2017) showed that whether fungal necromass was melanized or non-melanized affected the structure of the microbial decomposer communities. Thus, melanized ECM fungi act as ecological engineers of the forest soil impacting both C cycle and soil microbial composition. Our results suggest that *H. bicolor* could be an important and persistent species contributing to fungal biomass production in young nutrient-rich peatland forests like Rich1.

On the other hand, long exploration type ECM fungal genera *Piloderma* and *Suillus* were typical of the nutrient-poor sites characterized by higher C/N-ratio, lower P amounts and lower soil pH. These results are consistent with earlier findings that *Suillus* is a common genus in pine dominated boreal forests with high C/N-ratio (Sterkenburg *et al.*, 2015) and that species of *Piloderma* are known to favour under low-N conditions (Högberg *et al.*, 2014). *Piloderma* may contribute largely to cycling of critical nutrients in nutrient-poor peat since it is known to be capable of releasing organic N and delivering it to its tree host (Heinonsalo *et al*., 2015) and possess great potential to acid phosphatase production enabling mobilization of P (Velmala *et al*., 2014). Furthermore, compared to *Suillus* and

Lactarius genera, *Piloderma* in tree seedlings has been shown to accumulate more N into the roots while getting C as a reward by the plant (Sarjala and Potila, 2005).

Interestingly, the ECM fungus *Paxillus involutus* which was very common in almost all peatland sites, especially in Rich1 and Poor1 in the very beginning of the growing season, may play a dual role in peatland forests since it was recently shown to liberate organic N from soil organic matter via hydroxyl radicals produced by the Fenton reaction (Op de Beeck *et al.*, 2018). This ability to release N from soil organic matter and P at low concentrations (Colpaert *et al.*, 1999) might give *Paxillus* a competitive advantage over others in early stages of fungal colonization, especially in organic rich peatlands. As *P. involutus* is also known for rapid and explorative mycelial growth (Agerer, 2001), it might be a putative candidate for the relatively higher EMM fungal biomass production in both Rich1 and Poor1 sites during the first summer months. Another functionally interesting and abundantly found ECM fungal sequences were belonging to the genera of *Cortinarius;* several species have been shown to produce Mn-peroxidases enabling decomposition of N from complex organic matter (Bödeker *et al*., 2014). A few ECM fungal genera showed specificity to a certain sampling depth or recovery time of the bag. For example, *Tylospora and Tomentellopsis* were more common in the upper soil horizon (0-15 cm) whereas *Russula* and *Piloderma* were more common in the deeper soil horizon (15-30 cm). Since genus *Russula* have also previously been commonly observed from various types of drained boreal peatland sites, we suggest that its prevalence in the ECM fungal community in peatland forests (Peltoniemi *et al*., 2009, 2012, 2015) may be due to the large species-specific variation across different types of peatlands (see Table S4). Furthermore, *Tomentellopsis* was also more abundant in mineral-soil forest sites compared to others, indicating that it would prefer more mineral soils than the organic ones. It seems that vertical distribution of ECM species did not follow any previously observed trends, probably because in peatlands ECM and fine roots of trees follow WT rather than stratification of podzolic soil layers (Lindahl *et al*., 2007).

Peatland forest site type affects the magnitude of saprotrophic fungal biomass production and community composition

Hypothesis 4 was supported by our findings, although site explained only 10 % of the variation in saprotrophic fungal biomass production. Fungal biomass production in litter was higher in the Rich1 site if the values were converted to unit-area basis and assuming an even cover of litter across the sites, which is highly unrealistic, the values would correspond to 2.0 T ha^{-1} $month^{-1}$ in Rich1 vs. 1.6 T ha^{-1} $month^{-1}$ in Poor1. Even though these values are not comparable to the values estimated for EMM production, they indicate a very high production potential for saprotrophic fungi. This should not be surprising in a case where relatively easily decomposed litters were used, and where a high proportion of the soil itself is organic matter. Further, according to hypothesis 4 the fungal community composition showed differences between the Rich1 and Poor1 sites. Thus, a specific saprotrophic fungal community may be responsible for the higher fungal biomass production in the more nutrient-rich environment. Lower production in the nutrient-poor site may also indicate increased turnover of saprotrophic fungal biomass, if saprotrophic mycelia attracted other decomposers to rapidly recycle N bound in biomass in the nutrient-poor habitat (Brabcová *et al*., 2018). Interestingly, trenching in the Poor1 site resulted in less biomass in the deeper peat layer, whereas in the both sites it resulted in more biomass in the surface layer. The result may be an indirect evidence of a phenomenon referred to as the 'Gadgill effect' (Gadgil and Gadgil, 1971, 1975), where saprotrophic fungi may benefit from increased resources due to decreased competition with mycorrhizal fungi. Though, the Gadgil effect has recently been shown to be substrate-specific and thus case-dependent in mineral boreal forests (Sietiö *et al*., 2019).

Although a major portion (80%) of the fungal representatives was observed across both sites, a closer look at the saprotrophic fungal community and functional guilds may explain the differences between sites. For instance, *Hyaloscypha variabilis* which showed correlation to nutrient-poor conditions is a particularly common ERM fungi known to form associations with several species of Ericaceae and act as an endophyte in ECM roots of northern conifers (Grelet *et al*., 2010; Vohník *et al*.,

2013). Interestingly, a recent study verified that *H. variabilis* has a large gene repertoire for cell-wall and membrane degrading enzymes to switch from a symbiotic to a saprotrophic lifestyle (Martino *et al*., 2018). Another fungal representative, *Trichoderma viride,* showed correlation to lower N content and pH. Genus *Trichoderma* is widespread and can be easily found in soil, on decaying wood and contains opportunistic mycoparasites feeding on other fungi (Chet *et al*., 1998).

Rich1 had more unique genera which represented more diverse functional fungal groups. This may indicate that a nutrient-rich site has a functionally more diverse fungal community pool to respond to changing conditions, e.g., a sudden input of fresh organic matter, which shows as increased biomass production. Presence of a fungal representative affiliating to the wood-decomposing saprotrophic white-rot genus *Hypochinicium* was typical of Rich1 and correlated positively with iron content, which was one of the strongest factors separating fungal communities between sites. The soil of Rich1 was especially rich in Fe, deriving from its past as a wet site before drainage (see Westman and Laiho, 2003).

Both sites appeared to contain site-specific fungi with similar functions, but from different fungal taxa. For example, Rich1 had dung and wood saprotrophic (*Ascobolus)* and wood decomposing white rot taxa (*Hypochinicium)*, while Poor1 had fungi with similar broad functions related to dung and wood saprotrophs, including white and soft rot decomposers (family Pyronemataceae, genera *Sordaria* and *Gymnopilus)*. Typical for both sites were also r strategists including mostly yeasts and molds; both sites seemed to have a specific niche for Hypocreales (*Trichoderma* species) and Poor1 for Mucorales (*Mucor*) and Saccharomycetales (*Sugiyamaella*) found commonly in boreal forest soil (Sterkenburg *et al*., 2015). These results might indicate functional redundancy, but we cannot exclude a possibility that different fungal taxa showing specificity for studied sites would be a result of adaptation to certain fertility requirements.

Conclusion

The fungal in-growth mesh bag method was used for the first time to estimate the impact of site fertility on EMM fungal and saprotrophic mycelia production and ECM and saprotrophic fungal community composition in typical boreal peatland forest sites. The method that was initially designed for mineral soils seems to work well also for peat soils. Nutrient-poor conditions and a lower water table level in the peatland forest site in general predict higher EMM fungal biomass production. On the contrary, nutrient-rich conditions predict higher saprotrophic fungal biomass production. Both mycorrhizal and saprotrophic fungal community composition differed according to site fertility. We suggest that different ECM fungal communities and exploration types between peatland forest types may explain the differences in mycelial biomass production. The results suggest that the melanin-rich and slow-decomposing genus *Hyaloscypha* may be responsible for the high EMM fungal biomass in young nutrient-rich peatland forest. In addition, fungi with different life-strategies belonging to genera *Lactarius* and *Laccaria* may be important as well in nutrient-rich conditions. In contrast, species of *Piloderma* may have importance in releasing organic N and P in nutrient-poor conditions. *Paxillus involutus* and *Cortinarius* sp. may be an important generalist contributing to organic N release in all sites irrespective of fertility, especially during the early summer months. Functionally more diverse litter-inhabiting fungal community together with decreased competition of ECM fungi after trenching may be responsible for the higher fungal biomass in nutrient-rich compared to nutrient-poor conditions. Since our results clearly showed that site fertility affects fungal biomass production and community composition, they might be linked to studies estimating soil C balance in peatland forests. Therefore, one reason for previously reported soil C loss from nutrient-rich drained peatland forests (Ojanen *et al*., 2013) could be a functionally diverse and active saprotrophic fungal community, which enables fast decomposition. In contrast, in nutrient-poor peatland forests the ECM community which produces recalcitrant necromass could contribute to slow decomposition and positive soil C balance (Ojanen *et al*., 2013; Minkkinen *et al*., 2018).

Experimental procedures

Study sites

The study was done in four drained peatland forest sites located in southern Finland. The sites included two Norway spruce (*Picea abies)*, dominated nutrient-rich forests, Lettosuo (Rich1) in Tammela (60°39'N, 23°57'E) and another site (Rich2) in Orivesi (61°80'N, 24°30'E) in the vicinity of the Lakkasuo peatland complex. The two Scots pine (*Pinus sylvestris)* dominated nutrient-poor sites were Kalevansuo (Poor1) in Loppi (60°39'N, 24°22'E), and another (Poor2) in Orivesi in the Lakkasuo peatland complex (61°79'N, 24°31'E, respectively). Rich1, originally a herb-rich sedge birch-pine fen, was classified as *Vaccinium myrtillus* type II, Rich 2, originally a *Vaccinium myrtillus* spruce swamp, as *V. myrtillus* type I, and both Poor1 and Poor 2, originally dwarf shrub pine bogs, as dwarf-shrub type I according to Laine *et al.* (2012). The tree stand basal area and main ground vegetation for all sites is described in supporting information table ST1.

The sites were drained in the 1960's or early 1970's. In Rich1, the dominant pine tree storey was harvested in March 2016 before our experiment and uneven spruce-dominated stand with a mixture of pubescent birch (*Betula pubescens)* was retained (Korkiakoski *et al*., 2020). Other sites had tree stands typical of the site types: Rich2 a mature spruce stand and Poor1 (for further site information, see Lohila *et al*., 2011) and Poor2 mid-rotation pine stands. Average annual water-table levels estimated from continuous monitoring data for year 2016 for Rich1, Rich2, Poor1 and Poor2 sites were 37 ± 6 (mean ± SD), 31 ± 2, 30 ± 3, and 25 ± 1 cm below the peat surface, respectively. Nutrient elements and soil pH were measured from a separate set of peat samples taken 5–15 and 15–25 cm below the moss layer (Table 1). Total C and N were determined from air-dried samples with a LECO CHN-1000 analyser, and the concentrations of other elements with an ICP-emission spectrometer (ARL 3580) using dry ash dissolved in hydrochloric acid.

Two mineral-soil forest sites located in the vicinity of the Lakkasuo peatland complex in Orivesi were included in the experiment for comparison: nutrient-rich Norway spruce dominated site (RichM)

and nutrient-poor Scots pine dominated site (PoorM). EMM production in these sites was measured earlier by Yiyang *et al*. (unpublished data). We repeated the measurements to exclude the potential impact of annual variation in the results.

Sandbag method to determine EMM biomass and ECM fungal community

To estimate the biomass production of EMM of ECM fungi and their community composition, five replicate in-growth mesh bags (6 × 11 cm) were filled with approximately 125 g of acid washed quartz sand. The bags were buried at two depths (0-15 cm, 15-30 cm) in all four peatland forests and the two mineral-soil forests in the end of May 2016, and recovered after 2 (late July), 5 (October), and 12 months (late May 2017).

Recovered sandbags were cleaned of external dirt and gently opened with scissors. Homogenic sub-samples were ensured by mixing several spoonsful of sand all over the bag and immediately stored at -20°C for ergosterol extractions. The remainder of the sand was washed as described in Peltoniemi et al. (2015) and the harvested mycelia were frozen at -20°C for DNA extractions. A separate sample of sand or litter from each bag was dried at +105°C to determine the dry matter content. Ergosterol was extracted and measured from homogenized sub-sample by-taking 3 g of sand as described by Peltoniemi *et al.* (2015). We used a conversion factor, 3 mg ergosterol concentration corresponds to 1g fungal biomass, to calculate the fungal biomass production estimates from ergosterol concentrations (Salmanowicz and Nylund, 1988; Ekblad *et al.*, 2016). The final EMM biomass production from the sandbags is reported as kg fungal biomass ha^{-1} $month^{-1}$. For EMM biomass we accounted for sand density (1.45 g/cm^3), and the soil depth covered by the bag (ca. 10 cm), and the obtained value per cm^2 was scaled per hectare. Finally, EMM biomass production values from sandbags with different recovery times were converted to production per month.

Litterbag method to determine saprotrophic fungal biomass and community

To estimate the biomass production of saprotrophic fungi and their community composition, five replicate in-growth mesh bags were filled with approximately 11 grams of either sterilized corn or wheat litter. They were placed into trenched subplots in the Rich1 and Poor1 peatland forest sites, at two depths (0-15 cm, 15-30 cm) at the end of May 2016 and recovered after 5 months (October 2016). Initially, litters from the C4 plant (corn) and the C3 plant (wheat) were chosen to evaluate whether isotopic patterns could aid the estimation of saprotrophic fungal biomass production. In the end, however, we did not have enough material for isotopic measurements. To avoid non-saprotrophic fungi entering the bags, trenching was conducted just prior to installing by cutting all the root connections to the depth of 40cm with a chainsaw around a 1 × 1 m^2 area and surrounding the subplots with a herbicide-free root barrier fabric (polypropylene 220g/m^2). Control litterbags without trenching were buried outside the trenched subplots in a corresponding manner.

Recovered litter bags were cleaned for external dirt and sub-samples of litter were immediately stored at at -20°C for DNA and ergosterol extractions. Dry matter contents and ergosterols were determined as previously described for the sand material. The biomass production of saprotrophic fungi from the litters is reported as mg fungal biomass g^{-1} (litter dry weight) $month^{-1}$.

Chitin analyses for 12-month sandbags

The procedure for chitin-derived glucosamine extraction is described in detail by Adamczyk *et al.* (2020). The fraction of living fungal biomass (% of total) was calculated with the formula: $100(14/s - c)/(1 - c)$ where 14 is the chitin:ergosterol ratio in living cells, s is the chitin:ergosterol ratio of the sample, and c is the relative ergosterol concentration in inactive fungal cells which was assumed to be 10% (Ekblad *et al.*, 1998).

DNA extraction, sequencing and sequence data processing

DNA from freeze-dried sand was extracted with a NucleoSpin Soil kit (Macherey Nagel, Germany) and from litter with a NucleoSpin Plant kit (Macherey Nagel, Germany) according to the protocols of the manufacturer. DNA concentrations were measured with a Cubit fluorometer (ThermoFisherScientific, US) and DNA samples were sequenced by an Illumina Miseq platform at the Biotechnical Institute of Helsinki University. The ITS2 region was amplified in a 2-step PCR using the primers ITS4 (White *et al.*, 1990) and gITS7 (Ihrmark *et al.*, 2012) containing partial TruSeq adapter sequences at the 5'end. The first PCR was done in two replicate 25 µl reactions using Phusion Hot Start II polymerase (Thermo Fischer) and cycling conditions consisted of an initial denaturation step at 98°C for 30s, followed by 15 cycles at 98°C for 10s, 55°C for 30s, 72°C for 10s, and a final extension for 5 minutes. After PCR the two replicates were combined and treated with Exonuclease I (Thermo Scientific) and Thermosensitive Alkaline Phosphatase (FastAP; Thermo Scientific). A second PCR was performed with full-length TruSeq P5 and Index containing P7 adapters and 1-5 µl from the first PCR as template. Cycling conditions were similar to the first amplification but with 18 cycles and 50µl reactions with no replicates. Final purification was performed with Agencourt® AMPure® XP magnetic beads from Agencourt Bioscience (Beckman Coulter Inc, MA, USA). DNA concentration and quality were verified with Qubit and Fragment Analyzer (Advanced Analytical), respectively. The final PCR fragments were pooled in equal concentrations and run on a MiSeq Sequencer (Illumina) using v2 600 cycle kit paired-end (325 bp + 285 bp).

Quality filtering and removal of artifacts, primer-dimers and primers from raw sequence reads was conducted with the PipeCraft 1.0 pipeline (Anslan *et al*., 2017). Raw fungal ITS sequence reads were processed according to the manual as described by Soinne *et al*. (2020). OTUs that had affiliation other than fungi and singleton OTUs were removed from the data. Raw ITS sequence data is deposited in the sequence read archive (SRA) of NCBI/EMBL database under the BioProject id PRJNA586760 with the accession numbers SAMN13166700-SAMN13166799. All the downstream analyses were conducted from sequence reads that were normalized with geometric mean of pairwise ratios (GMPR) method

(Chen *et al*., 2018) from OTUs that matched with representative taxa in the ITS2 database (sh_genral_release_dynamic_01.12.2018.fasta) from UNITE (Nilsson et al., 2018) representing unique fungal phylotypes.

Statistical analyses of the fungal biomass data

The response of EMM fungal biomass production (kg ha^{-1} $month^{-1}$) to site and soil characteristics was analyzed with linear mixed models using MLwiN 2.26 software considering the hierarchical structure in the data (Rasbash *et al*., 2019). Models including only constants were used for estimating the mean production values. For the ECM fungal (sandbag) data, site (*l*; 4 sites), location within site (*k*; 5 locations per site), depth level within location (*j*; 2 levels: 0-15 cm and 15-30 cm), and recovery time within depth level, location and site (*i*; 2, 5, and 12 months after installation), were coded as hierarchical levels. The basic form of the model was

$$\mathrm{Fbm}_{ijkl} = \beta_{0ijkl} \cdot \mathrm{constant} + \beta_{(1\text{-}n)} \cdot x_{(1\text{-}n)l\text{-}ijkl}$$

where the value of the constant, β, is allowed to vary at all hierarchical levels (*ijkl*) resulting in the residuals also being estimated at each level as f_{0l}, v_{0kl}, u_{0jkl}, and e_{0ijkl}, whereas the parameters *P* for variables *V* vary at different levels depending on the level at which the variables were measured (e.g., site type was measured at the site level, while average peatland WT was available at the recovery time level). The residuals form the random part of the model and are expected to be uncorrelated and follow normal distribution, so that it is sufficient to estimate their variances only.

Potential explanatory variables for the peatland sites were peat characteristics shown in Table 1 (individual characteristics plus element ratios to C and N were tested), and other variables shown in Supporting Information Table ST1 including site type (identified as Rich and Poor), tree stand basal area, cover of different plant functional types in the ground vegetation, average WT for the recovery

times as well as for a 13 month period including the month preceding installation, and fine root production estimated for 0-10 cm and 0-50 cm layers based on root ingrowth core data collected by Raija Laiho and coworkers (Lampela *et al*. unpublished data; measured as in Bhuiyan *et al*., 2017).

Some variables identified as hierarchical levels were also tested as explanatory variables (depth, recovery time = length of the burying period of the bags). In such cases their residual variances generally became non-significant. Also, if the effect of some level was well explained by the fixed part, the variance of that level approached zero. All levels were nevertheless retained in the model structure to facilitate direct comparison of the goodness of fit for different models using the -2*loglikelihood measure (Rashbash *et al.*, 2019).

In several models only the contribution of the last hierarchical level, recovery time, to the residual variance was significant. This basically indicates non-significant autocorrelation in the data, but it may alternatively be caused by the relatively small number of observations. We chose to report the results of the full mixed models in each case, even though in the case of non-significant variance components, a simple regression analysis with only the fixed part of the models could also be applied.

The litterbag fungal biomass data [mg g^{-1} (litter dry weight) $month^{-1}$] was analyzed similarly, but the models lacked the repeated measures aspect as there was just one recovery time. Also, since there were only two sites in that data set, we were able to analyze only the impacts of site, depth, litter type and trenching treatment. All other variables in the data set would simply describe the difference between the two sites.

Significance of differences in the average amount of living fungal biomass in sandbags after 12 months between sites and depths was tested with analysis of variance (ANOVA) with R-studio version 1.1.442 (RStudio Team 2016) with R version 3.4.4 or 3.5.2 (R Core Team, 2018). Tukey's HSD (Honest Significant Differences) post-hoc test was used to reveal statistically different groups.

Analyses to investigate fungal community composition

For all community analyses we used R-studio version 1.1.442 (RStudio Team 2016) with R version 3.4.4 or 3.5.2 (R Core Team, 2018). Significant differences in OTU numbers, i.e., fungal richness, between sites, depths and recovery time of the sandbags, or sites, depths, litter types and treatments (trenched or not) from the litterbags was tested with analysis of variance (ANOVA). Tukey's HSD (Honest Significant Differences) post-hoc test was used to reveal statistically significantly different groups. Shared and unique OTUs between sites and depths from the sandbags or sites, depths, litter types and treatments (trenched or not) from the litterbags were visualized for 2, 5 and 12 month data separately by applying R packages venn (Chen, 2018), VennDiagram (Dusa, 2018) and car (Fox and Weisberg, 2011). FUNGuild, the online application tool, was used to detect functional information, fungal guilds of OTUs (Nguyen et al., 2016). We did permutational multivariate analysis of variance (PERMANOVA) using distance matrices with function adonis in package vegan (Anderson, 2001) to test the effect of site, depth and recovery time on sandbag fungal community composition, and the effects of site, depth, treatment and litter type on litter bag fungal community composition. We also conducted nonmetric multidimensional scaling (NMDS) with stable solution from random starts, axis scaling and species scores with function metaMDS from vegan using the Bray-Curtis dissimilarity index and plotted the NMDS with fitted environmental variables with function envfit from vegan (Oksanen *et al*., 2018). Paired comparisons to identify fungal indicator OTUs were conducted with differential abundance analysis using DESeq2 (Love *et al*., 2014). This produced a list of significant fungal representatives which differed in their abundances in compared cases when the ratio of the difference between final value and the initial value over the original value (log2FoldChange) is > 1.8 (adjacent p >0.05). Nonparametric Spearman's rank-order correlation analyses (function cor.test in R) were conducted for the dominant fungal representatives from both sand- and litterbags with fungal biomass, soil pH and nutrient data

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Author contributions

R.L., H.F., K.M., K.P., Ta.Pe. and Ti.Pe. designed the research; K.P., R.L. analyzed the data and drafted the manuscript; Ti.Pe. conducted the field work; S.A. conducted the chitin analyses; T.S. performed the ergosterol analyses; all authors contributed to interpretations of the data and writing the manuscript.

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Figure legends

Fig 1. Fungal biomass production in peatland forest and mineral-soil forest sites at two depths (0−15 cm, 15−30 cm) from a) in-growth sandbags targeting ECM fungi recovered after 2, 5 and 12 months and from b) litterbags targeting saprotrophic fungi recovered after 5 months. Bars represent standard error of means. Abbreviations: corn, c, and wheat, w, litter types.

Fig 2. Nonmetric multidimensional scaling (NMDS) plots showing differences in fungal community composition from sandbags targeting ECM fungi between four peatland forest (Rich1, Rich2, Poor1, Poor2) and two mineral-soil forest sites (RichM, PoorM). Data originating from 2, 5 and 12 month and 0-15 and 15-30 cm sampling depth bags have been combined. Ellipses show the confidence level of 95%. Arrows show environmental variables that fit the x-y data of the NMDS significantly ($P > 0.05$).

Fig 3. The heatmap of the most dominating taxa in sandbags (proportion of the normalized reads at least in 5% in the sample type (n=3). Functional guilds: ECM, ectomycorrhiza; SAP, saprotroph; PAT, pathogen; SYM, symbiotroph; END, endophyte.

Fig 4. Nonmetric multidimensional scaling (NMDS) plots showing differences in fungal community composition from litterbags targeting saprotrophic fungi between nutrient-rich (Rich1) and nutrient-poor (Poor1) peatland forest sites. Ellipses show the confidence level of 95%. Arrows show environmental variables that fit the x-y data of the NMDS significantly ($P > 0.05$). See abbreviations in Fig 1.

Fig 5. The heatmap of the most dominating taxa in litterbags (proportion of the normalized reads at least in 5% in the sample type (n=3). See abbreviations in Figs 1, 2 and 4.

Table 1. Soil pH and element concentrations in the surface peat of the peatland forest sites.

Site	Depth	soil pH	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B	N%	C%	CN	NP
Rich1	5-15 cm	4.1	0.95	0.41	3.0	0.40	8.2	79	36	8.4	2.8	2.0	50	25	2.1
Rich2		3.9	0.68	0.79	3.8	0.57	1.8	274	34	5.3	3.1	1.5	49	41	1.9
Poor1		3.8	0.65	0.66	2.7	0.49	1.1	88	61	5.5	2.3	1.2	50	34	2.1
Poor2		3.9	0.65	0.63	2.6	0.54	0.6	108	49	4.3	2.0	1.2	49	40	1.9
Rich1	15-25 cm	4.1	0.61	0.06	2.0	0.13	4.9	10	3	3.9	1.0	2.2	55	25	3.6
Rich2		4.1	0.52	0.14	3.9	0.41	8.4	14	6	5.3	1.4	2.0	54	56	3.5
Poor1		3.8	0.27	0.08	1.1	0.25	2.3	3	10	1.1	0.9	1.0	53	27	3.8
Poor2		4	0.32	0.06	1.7	0.30	0.2	3	6	1.3	1.1	1.2	48	41	3.7

Table 2. The model best explaining the variation in the fungal biomass production (kg ha^{-1} $month^{-1}$) in the sandbags. Fixed part includes the significant explanatory variables, their parameters, and parameter standard errors in parentheses. The random part includes the variance components of the different hierarchical levels identified in the data after fitting the model (see Experimental procedures). The model explained 41.6% of the initial variation (accounting for also the non-significant variance components). The production depended on site type (Poor vs Rich), average WT during the recovery time, length of the recovery time, and soil N:P ratio.

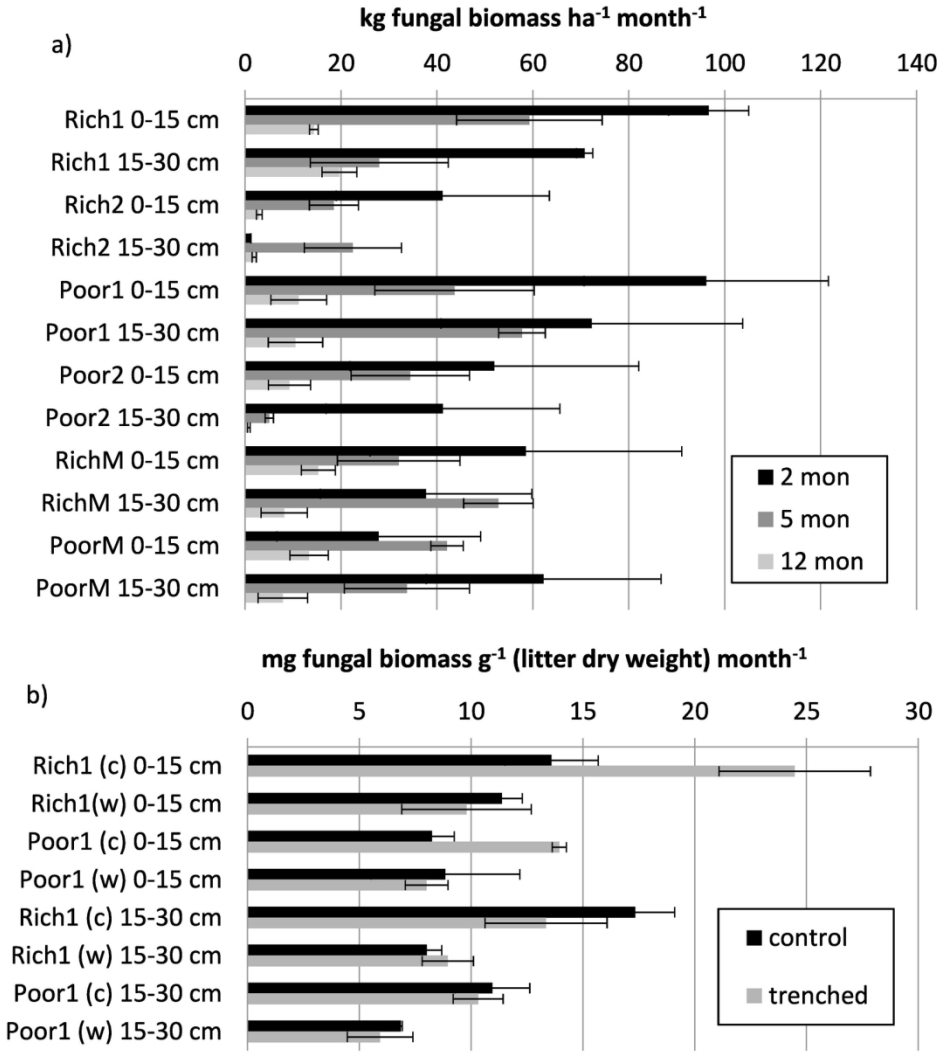
Fixed part		Random part	
Constant	-98.8 (38.0)	Site	0.0 (0.0)
Poor[1]	39.3 (9.4)	Location	11.3 (77.8)
WT (cm below surface)	5.1 (1.0)	Depth	4.9 (118.7)
N:P	-7.6(3.6)	Recovery time	986.6 (156.9)
5-month recovery time[2]	-30.6 (7.1)		
12-month recovery time[2]	-45.6 (7.1)		

[1] as compared to the reference category Rich
[2] as compared to the reference category 2-month recovery time; recovery time was coded as three categorical variables, since that improved the model as compared to coding time as a continuous variable, indicating that the temporal pattern was not quite linear

Table 3. The model best explaining the variation in the fungal biomass production [mg g^{-1} (litter dry weight) $month^{-1}$] in the litterbags incubated at two peatland forest sites, Rich1 and Poor 1. Fixed part includes the significant explanatory variables, their parameters, and parameter standard errors in parentheses. The random part includes the remaining variance components of the different hierarchical levels identified in the data after fitting the model (see Experimental procedures).

Fixed part		Random part	
Constant	16.2 (0.8)	Site	0.0 (0.0)
Site[1] x Trenching	4.6 (1.0)	Location	0.2 (1.3)
Depth[2]	-5.4 (0.9)	Depth	12.1 (2.5)
Litter type[3]	-6.3 (0.9)		

[1] difference of the nutrient-rich site from the nutrient-poor site
[2] difference of depth 15-30 cm from the depth 0-15 cm
[3] difference of the wheat litter from the corn litter



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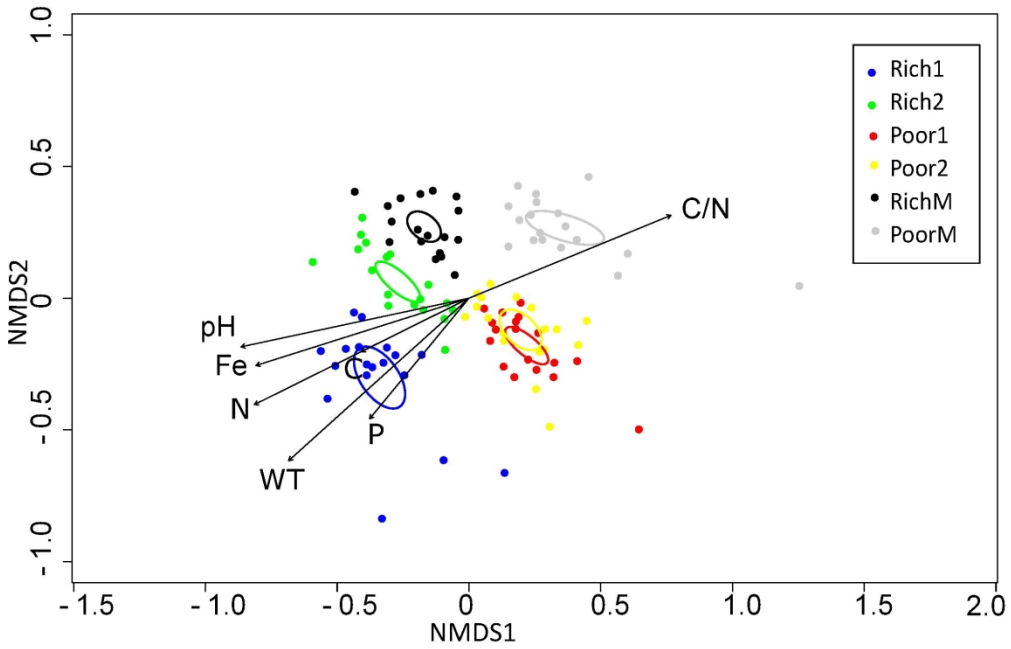

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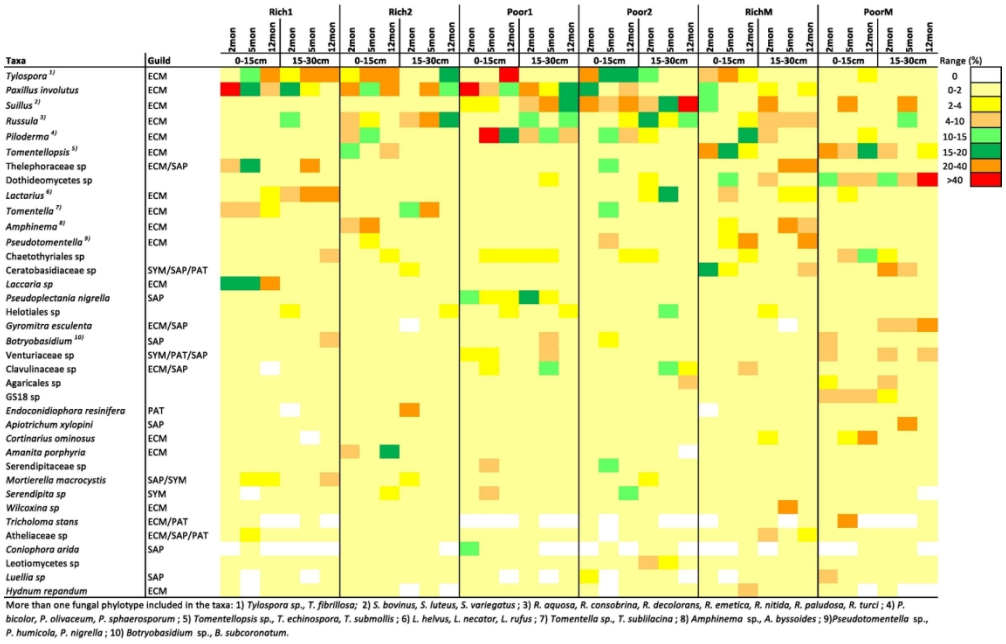

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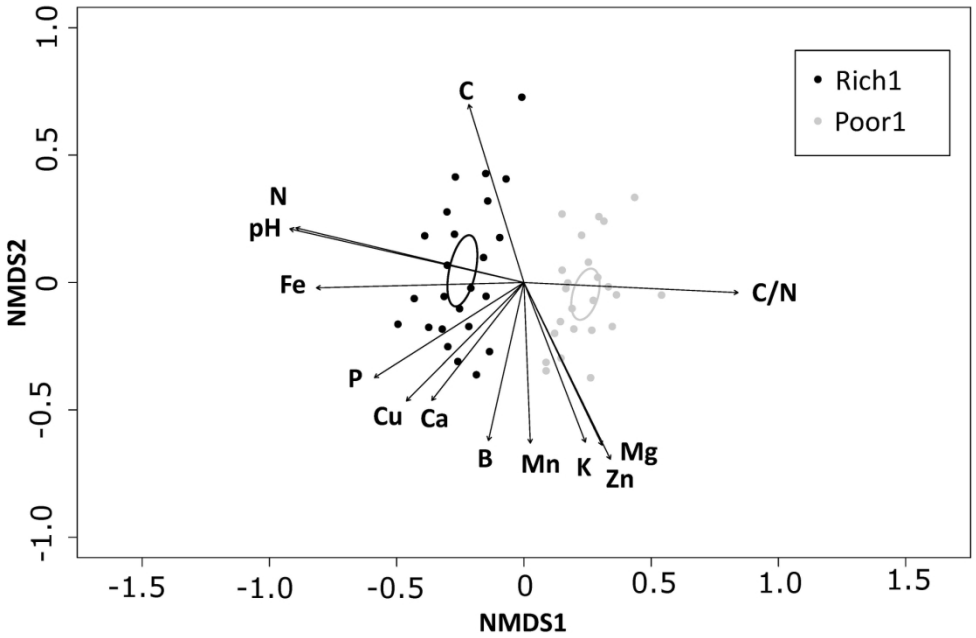

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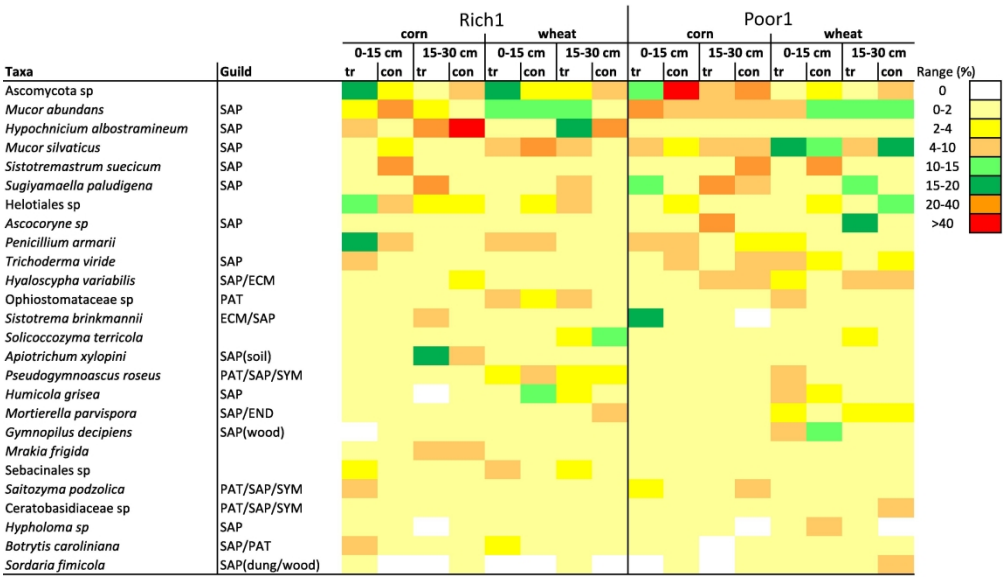

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